

podoplanin using ELISA, Western-blot, and flow cytometry. Using IHC analysis, LpMab-7 showed high reactivity against osteosarcoma tissues compared with NZ-1 mAb. Furthermore, LpMab-7 detected podoplanin expressed in metastatic lesions of osteosarcomas. Of interest, podoplanin expression at metastatic lesions was higher compared with primarily lesions in 3 of 4 cases with lung metastasis.

Discussion: We investigated podoplanin expression by IHC using LpMab-7 mAb against 16 osteosarcoma tissues, four of which have pulmonary metastatic lesions. Although 3 of 4 metastatic lesions showed higher podoplanin expression than primary ones, more cases should be examined to conclude the association between podoplanin expression and osteosarcoma metastasis. Because LpMab-7 has high sensitivity against podoplanin, LpMab-7 mAb is expected to be useful for molecular targeting therapy and a metastatic marker for osteosarcomas.

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INTRODUCING A NOVEL SURGICAL TOOL TO FACILITATE IM NAILING – FEMORAL ANTEGRADE STARTING TOOL (FAST)

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Introduction: The current standard of care in lower extremity long bone fracture stabilization is closed intramedullary nailing (IMN). The surgical protocol associated with this surgery is well defined. Yet, challenges arise that impede the surgical workflow and lead to frustration in the operating room. Specifically, two surgical steps of entry point selection and reduction have been identified in the literature as the most challenging steps. Both of these steps utilize 2D fluoroscopic imaging to guide 3D alignment. Challenges arise when the alignment in one plane is lost while adjusting the alignment in the perpendicular plane. This leads to unpredictable repetition of activities which can be time consuming and frustrating. The primary aim of this study is to develop an innovative surgical instrument to facilitate the entry point selection.

Methods: Design requirements were identified by shadowing three surgeons performing eight IMN procedures in the operating room and conducting semi-structured interviews. Once a 3D model of the device was developed, a team, consisting of an experienced staff surgeon, a junior orthopaedic surgeon, and a mechanical engineer was consulted. Upon addressing the team comments, a potential device design was finalized and prototyped. The prototype was shown to the team to ensure the ease-of-use of the device as well as its functionality. However, multiple design improvements were made to optimize the ease-of-use of the device. The prototype of the new design was manufactured and tested on a synthetic bone with surrounding foam to simulate soft tissue. Three surgeons conducted the surgery under standard operating room conditions and provided feedback. The above process allowed the inventors to finalize the device's patent-pending design and establish the device use protocol.

Results: The proposed design consists of a fixed frame and a rotatable multicannulated arm to assist in guide wire insertion in femoral IMN. The device is initially placed at the approximate IMN entry point location on the femoral head under fluoroscopic image guidance. An anterior-posterior (AP) image is taken to align the rotatable arm with the intramedullary canal of the femur. Once the 2D alignment is satisfactory, the device is temporarily fixed to the femoral head via two pins. Based on the AP image a K-wire is placed into one of the three cannulated entry point AP positions. A subsequent lateral (or oblique-lateral) image is then taken to identify the correct 3D trajectory for accessing the intramedullary canal. The device can then be adjusted within the selected plane to match the correct trajectory via fixed rotation of the arm. The entry point can also be adjusted via selection of an alternate lateral cannula. The K-wire is then advanced through the cannulated guide into the intramedullary canal.

Conclusion: This simple device represents a novel surgical tool for use in IMN. In contrast to the current entry point selection activity cycle (which may include the acquisition of multiple AP and lateral images, patient repositioning and inaccurate drilling), use of the device ensures maintenance of the 2D alignment obtained in the AP plane while adjusting positioning in the perpendicular (lateral) plane.

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THE EFFECT OF ANTI-TNF INHIBITORS ON INNATE IMMUNE SYSTEM IN SYNOVIAL TISSUES IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Potential biologic therapies have been developed for the prevention of joint destruction in rheumatoid arthritis (RA) patients. However, 20–30 % of RA patients using biologics including anti-TNF inhibitors are non-responders or only minor improvement. Residual inflammation suggests a risk for progression of joint destruction. Furthermore, recent evidence has strongly suggested that the onset

and progression of RA depend on many different factors including innate immune sensors, such as Toll-like receptors (TLRs), participate in the induction of innate inflammatory response, and also following adaptive and/or autoimmune responses play an important role in RA inflammation.

Objectives: The aim of this study was to investigate the immunoinflammatory cells, including Toll-like receptor (TLR)-equipped cells, in synovial tissue samples from RA patients on anti-TNF inhibitors compared to patients with treatment of conventional synthetic disease-modifying antirheumatic drug (csDMARD).

Methods: Immune-inflammatory cells were evaluated in RA synovitis in patients with anti-TNF group [n=20 (etanercept 14, infliximab 6)] or csDMARD group (n=20) by immunohistochemical and immunofluorescence study. Mean duration of affection by RA of anti-TNF group and csDMARD-group was 8.3 years and 11.5 years, respectively. Period of anti-TNF group was 14 months. Mean CRP level of anti-TNF group and csDMARD-group was 15 g/dl and 22 g/dl and that of DAS28-CRP score (4) of anti-TNF group and csDMARD-group was 4.0 and 4.6 at collecting their samples, respectively. CD3 (T cells), CD20 (B cells), CD68 (macrophages), S-100 (dendritic cells:DC) and TLR1 to 9 immunoreactive cells were counted in at least five ×200 light microscope fields in larger lymphoid infiltrates. The intensity of the inflammation was estimated using the Krenn histopathological grading system (grade 0–3).

Results: The grading scores of synovitis was both 1.7 in each group and correlated best with the T and B cells in the both groups (p<0.05). Interestingly, both T and B cell counts were lower in the anti-TNF than in the csDMARD group (p<0.05). In contrast, the C-reactive protein (CRP) and disease activity score DAS28-CRP did not show clear-cut correlations with the inflammatory grade of the synovitis. Similar numbers of cells immunoreactive for TLR-1 to TLR-9 were found in synovitis in both groups.

Conclusion: Patients clinically responding to anti-TNF inhibitor might still have the potential of moderate/severe local joint inflammation, composed in particular of and possibly driven by the autoinflammatory TLR+ cells.

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USE OF THE COLLABORATIVE CROSS GENE MINE MOUSE PHENOTYPE LIBRARY TO IDENTIFY NOVEL GENES REGULATING BONE MASS AND BONE ARCHITECTURE

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Background: It is well established that there is a strong genetic effect on bone mass, bone loss and fracture risk; however, the vast majority of genetic variance for osteoporosis-related phenotypes remains unexplained. Novel approaches are needed to better identify the genetic underpinning of osteoporosis and to develop an understanding of the physiological and pathological roles of genes identified in this process.

Subjects and Methods: We utilised the Collaborative Cross (CC) Gene Mine mice to identify genes associated with bone volume. We employed μ CT to scan hindlimbs of 940 CC mice across 56 strains incorporating multiple ages and genders where available for each strain, and generated data on variables including BV/TV, Tb.N, Tb.Sp, Tb.Th, SMI, DA and Ct.Th from reconstructed femur images using CTAn software. Genomapping was then performed to identify candidate genes responsible for bone volume. We also correlated the candidate genes with femoral neck BMD in human cohorts.

Results: Dozens of candidate genes associated with these variables in mice were identified based on our analyses. Based on linkage analyses of BV/TV, peak loci were found at chromosome 13 in female mice and chromosome 3 in male mice, respectively. The former locus harbours candidate genes like EphA5, Itga1, Pclo and Itga2, and the latter harbours candidate genes like Snx27 and Txnip. With comparison of young and old mice, peak loci were found at chromosome 11 and 17 in female mice, suggesting potential genes like Fam83g and Rnf112; whereas peak loci were found at chromosomes 2 and 12 in male mice, suggesting potential genes such as Apob, Slc7a15, Laptm4a and Matn3. DA analyses in female mice showed peak locus on chromosome 18, which harbours genes like Ska1, Myo5b, Lipg, Epg5, Setbp1 and Nfatc1.

Discussion and Conclusion: Among these candidate genes, five potential genes, namely Txnip, Nfatc1, Setbp1, Apob and Itga1, have been reported to be associated with femoral neck BMD in human subjects. Nfatc1 has been extensively explored in its role in the osteoclast fusion process. Txnip, which is linked to glucocorticoid-induced bone loss, is thought to be a strong candidate gene for bone mass. Setbp1, which is closely related to Schinzel Giedion syndrome and atypical CML, is hypothesised to produce a gain-of-function mutation which may result in reduced PP2A and subsequently enhanced osteoblastogenesis. Itga1 is highly expressed in osteoblasts and its loss has been identified to be correlated with impaired fracture healing, accelerated knee osteoarthritis and reduced BMD in humans. Apob is mainly responsible for carrying lipids. Mutations in this gene

have been found to cause familial hypobetalipoproteinemia, hypercholesterolemia and decreased BMD in humans. Some other candidate genes also show promising roles in the regulation of bone mass in animal studies. For instance, Pelota regulates the development of extraembryonic endoderm through activation BMP; Matn3 bind BMP-2 and inhibits downstream BMP-2 signalling. Further investigation will focus on the functional studies of selected genes like Txnlp and Setbp1 via gene knockdown. Ultimately, this approach promises to generate novel genetic targets for the development of osteoporosis treatments.

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FIBROBLAST GROWTH FACTOR RECEPTOR 3 DEFICIENCY DOES NOT IMPAIR THE OSTEOANABOLIC ACTION OF PARATHYROID HORMONE ON MICE

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Background: Previous studies showed that endogenous fibroblast growth factor 2 (FGF-2) is required for parathyroid hormone (PTH)-stimulated bone anabolic effects, however, the exact mechanisms by which PTH stimulate bone formation and the function of FGF receptors in mediating these actions are not fully defined. FGF receptor 3 (FGFR3) has been characterized as an important regulator of bone metabolism and is confirmed to cross-talk with PTH/PTHrP signal in cartilage and bone development.

Subjects and Methods: *Fgfr3* knockout and wild-type mice at 2 months of age and 4 months of age were intraperitoneally injected with PTH intermittently for 4 weeks and then the skeletal responses to PTH were assessed by dual energy X-ray absorptiometry (DEXA), micro-computed tomography (μ CT) and bone histomorphometry.

Results: Intermittent PTH treatment improved bone mineral density (BMD) and femoral mechanical properties in both *Fgfr3*^{-/-} and wild-type mice. Histomorphometric analysis showed that bone formation and bone resorption were increased in both genotypes following PTH treatment. PTH treatment increased trabecular bone volume (BV/TV) in WT and *Fgfr3*-deficient mice. The anabolic response in *Fgfr3*-deficient and wild-type bone is characterized by an increase of both bone formation and resorption-related genes following PTH treatment. In addition, we found that *Fgfr3* null osteoblasts (compared to wild-type controls) maintained normal abilities to respond to PTH-stimulated increase of proliferation, differentiation, expression of osteoblastic marker genes (*Cbfa1*, *Osteopontin* and *Osteocalcin*), and phosphorylation of Erk1/2.

Conclusions: Bone anabolic effects of PTH were not impaired by the absence of FGFR3, suggesting that the FGFR3 signaling may not be required for osteoanabolic effects of PTH activities.

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SEXUAL DIMORPHISM IN BONE MORPHOLOGY DURING GROWTH IN CHINESE

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Introduction: Puberty is a time of rapid matrix mineral accretion. Studies of pubertal growth in adolescents using high resolution peripheral quantitative computed tomography (HR-pQCT) suggest that there is transient cortical fragility due to delays corticalisation of growth plate trabeculae during puberty [1]. Chinese children have increased risk for forearm fracture and so we aimed to investigate cortical growth in healthy Chinese adolescents during puberty.

Subjects and Methods: 218 boys and 221 girls aged between 7 and 17 years old with no bone diseases were recruited. Maturity was assessed by self-reported Tanner staging with the guidance of technical staff. Images of non-dominant distal radius was obtained using HR-pQCT (XtremeCT, Scanco Medical) with the reference line at 5mm proximal to growth plate. Proximal 40 slices of 110 slices were analysed by StrAx1.0. Cortical cross-sectional area (CSA), porosity, volumetric bone mineral density (vBMD) and trabecular bone volume fraction (BV/TV) were measured and adjusted for total CSA. Matrix mineral density was also measured.

ANCOVA was used to identify sex difference in bone parameters after adjustment for age, height and body weight. One-way ANOVA and Dunnett's test were used to examine sex differences in bone parameters across puberty.

Results: Compared to girls with the same Tanner stage, boys were 0.6–0.9 years older and 6.7–11.3 kg heavier in Tanner 2 and 3, and 6.7–9.7 cm taller in Tanner 2 to 5. After adjusting for age, height and weight, boys still had 10.1–14.7% larger total CSA than girls in all Tanner stages ($p < 0.05$ at Tanner stage 1 and 5; the rest $p < 0.001$). The cortical and trabecular CSA were also larger in boys but the cortical CSA was similar to girls in Tanner stage 2 and 5 after adjustment. In Tanner stage 1, cortical thickness was 7.0% greater in boys ($p < 0.01$) but cortical porosity did not differ from girls. From Tanner stage 2, cortical thickness did not differ by sex while boys had 8.4–12.6% higher cortical porosity (all $p < 0.001$) and 0.5–1.4% lower matrix mineral density ($p < 0.05$ for Tanner stage 2, $p < 0.001$ for Tanner 3–5) after adjusting for age. In boys, 10.7 – 19.1% lower vBMD of entire bone and 16.7 – 20.4% lower cortical vBMD were found in Tanner 2 to 5 compared to girls. There was no sex difference in trabecular vBMD until Tanner stage 3, then boys had 14.7–23.3% greater trabecular vBMD after adjusting for age (all $p < 0.01$).

Conclusion: Total and cortical CSA increase during pubertal growth in both sexes and are larger in males. Cortical porosity was higher and matrix mineral density were lower in boys, perhaps because their skeletal maturing takes longer to complete.

References

[1] Wang et al., 2010, JBMR.

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EFFECT OF LOW-DOSE X-RAY IRRADIATION AND Ti PARTICLES ON THE OSSEOINTEGRATION OF PROSTHETICS

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Background: Wear debris is known to inhibit the activity of osteoblasts and induces inflammatory reaction, which may contribute to the aseptic loosening of prostheses. Low-dose irradiation (LDI) exhibits a positive effect on osteoblasts and inhibitory effect of inflammation. Here, we test the hypothesis that LDI can promote osseointegration and inhibit the inflammatory membrane formation in the presence of titanium (Ti) particles.

Methods: Twenty-four male New Zealand rabbits were randomly divided into the SHAM and 0.5 Gy groups. After the animal was anesthetized, sterile saline (0.2 mL) was injected into each cavity of the left femur (NS), while the right femur was injected with equal volumes of suspended endotoxin-free Ti particles (3.4×10^8 particles). The Ti implant was then inserted into the hole drilled on the femoral condyle. Two days later, both distal femurs of the animal were exposed to 0.5 Gy X-ray irradiation. The PINP concentration was determined at day 0, 2, 4, and 8 weeks after operation. Trabecular morphology around the implants 8 weeks after operation was assessed using micro-CT, then the maximum push out force of samples was assessed using biomechanics test. Bone histomorphology study without decalcification was performed 8 weeks after operation.

Results: At 8 weeks postoperation, the newly formed bone around the implant in the distal femur could be seen in all the groups. Ti particles injection significantly decreased, while 0.5 Gy irradiation increased DA of trabecula around the implant. The 0.5 Gy irradiation significantly increased Tb.Th, BMD, Tb.N, BV and BV/TV, while decreased SIM of trabecula around the implant. The PINP concentrations in both groups increased after irradiation and peaked 2 weeks later. The concentration of PINP in the 0.5 Gy group was higher than that in the 0 Gy group at week 2. Histologically, interface membrane formation could be seen around the implant in the 0 Gy–Ti group and the 0.5 Gy–Ti group. However, the thickness of the interface membrane in the 0.5Gy–Ti group was significantly thinner compared to 0 Gy–Ti group. Fluorescence scanning microscopy showed the extensive xyleneol and calcein labeling around the implant. Ti particles injection significantly decreased the MAR, MS and BFR, while 0.5 Gy irradiation significantly increased MAR. Furthermore, the 0.5 Gy irradiation significantly increased push out force for implants. However, there was no significant difference in peak push-out force between the 0 Gy–NS group and the 0 Gy–Ti group.

Discussion and Conclusion: The LDI can significantly improve osseointegration of prosthetic and stability of the prosthesis when there was no wear particles. Although promotion effects for bone formation induced by LDI can be counteracted by wear particles, LDI can significantly inhibit the interface membrane formation around the implant induced by wear particles. In conclusion, LDI may be useful for enhancing the stability of prosthesis when there are no wear particles and for inhibiting the interface membrane formation during the early stage of aseptic loosening in the presence of wear particles.

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